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# UV Spectrophotometric Determination of Luliconazole Semisolid Dosage Form

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**Abstract**

Luliconazole mostly prescribed drug for the management of superficial problems, the very simple, Fast, accurate and economical methods have been proposed for the determination of Luliconazole cream. Luliconazole was measured by using Uv spectroscopy method with the solution of mrthanol and purified water the linearity was found to be 0.9987 and the accuracy showed mean % RSD of 0.921776 and with total meam % RSD 1.10130 in intermediate precision, robustness %RSD 0.543539 all the paranmeters values were within standard limit thus Analytical method was validated according to ICH guideline for the determination of Luliconazole cream. The method was found to be precise and validated as per ICH guidelines.

***Keywords:*** UV Spectrophotometry; Luliconazole.

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## 1. Introduction

The superficial mycoses are global problems, which affect 20 to 25% of the world's population, leading to degradation of quality of life in terms of cosmetic deformity. Luliconazole is a broad-spectrum antifungal agent with imidazole group mostly prescribed for the treatment of dermatological problems like superficial mycoses. [1] the chemical name of Luliconazole is (2E)-2-[(4R)-4-(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile with molecular formula $C_{14}H_9Cl_2N_3S_2$, and molecular weight 354.28. Luliconazole is yellowish powder. The USFDA approved that the 1 % of Luliconazole cream is indicated for the topical treatment of interdigital tineapedis, tineacruris, and tineacorporis [2] The characteristic of drug is poorly water-soluble as well as high permeability. It is low toxic well tolerable, being both fungistatic and fungicidal with minimum inhibitor concentration, MIC 0.004– 0.008µg/ml for most of the dermatophytes [1, 5]. The mechanism of action of LNZ is inhibition of Cytochrome P450 2C19 [6].Luliconazole inhibit the enzyme Lanosterol demethylase which is required for the synthesis of Ergosterol, a major component of the fungus cell membranes [7]. In Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP), Luliconazole is not official drug. A Review of literature reported very few methods such as LC-MS/MS [8] method (toe nails) HPLC for related substances [9], HPTLC method for assay in formulation and biofluid [10], and a single UV spectroscopic method [11] (area under curve) for assay of Luliconazole. There is not a simple method reported for the detection of the drug in pharmaceutical formulation by UV spectrophotometry. So, this research tends to establish a simple, fast, accurate, precise, reproducible and economic method for assay of Luliconazole dosage forms, which can be used in quality control laboratories. This paper reports a study on the development of new validated UV- spectrophotometric methods for the quantitative determination of Luliconazole in creams. The developed methods were validated as per ICH guidelines

## 2. Materials and methods

Reference Luliconazole was obtained as gift sample from Time Pharmaceuticals (Mukundapur-5, vhaisakhori). Methanol (AR) grade was supplied by Thermo Fisher Scientific India Private Limited and Luliconazole cream form (cream, ) containing 1% w/w of Luliconazole was procured from local pharmacy. Sodium acetate was procured from Thermo Fisher Scientific India Private Limited. Throughout the analytical process distilled water was used.

### *2.1 Instrumentation*

A PerkinElmer UV-Visible spectrophotometer (λ 365) with an even set of 10 mm quartz cuvettes were used for measurement of absorbance for the analysis. All analytical weight measurements were done on a PRECISA (XB120A) electronic balance.

### *2.2 Preparation of Calibration curve*

Standard stock solution was prepared by transferring 10mg of Luliconazole into 10ml volumetric flask and it was dissolved with methanol. Working standard solution was prepared by taking 2.5ml of stock solution into a 25ml volumetric flask. The volume was made up to mark to produce 100µg/ml solution with methanol and

distilled water. From the above working solution 0.2ml, 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml were pipetted and transferred to 6 individual 10 ml volumetric flask and finally the volume was made upto the mark with diluent. Solutions were scanned from 200-400 nm UV range by using UV-Visible double beam spectrophotometer.

**Assay**

***2.3 Preparation of Standard Solution***

Dissolve 50 mg of Luliconazole WS in 30 ml of methanol, sonicate it to dissolve and add sufficient methanol to produce 50ml. Dilute 1 ml of the solution to 100 ml with methanol.

***2.4 Sample preparation***

Weigh accurately about 1 gm of the cream and dissolve in 70 ml methanol, sonicate for 10 minute and add sufficient methanol to produce 100 ml. filter the resulting solution with whatmann filter paper. Further dilute the 5 ml of filtrate solution to 50 ml with methanl.

***2.5 Procedure***

Measure the absorbance of both the standard and sample solution in a UV visible spectrophotometer having 1 cm pathlength at a wavelength of 296 nm against blank and calculate the content of Luliconazole using following formula.

***2.6 Calculation***

(Content of Luliconazole in % w/w)

$$\frac{\textbf{Sp absorbance}}{\textbf{Std absorbance}} \times \frac{\textbf{Wstd}}{\textbf{50}} \times \frac{\textbf{1}}{\textbf{100}} \times \frac{\textbf{100}}{\boldsymbol{Wsp}} \times \frac{\textbf{50}}{\textbf{5}} \times \textbf{Potency of std}\,\% \times (\textbf{100}\% - \textbf{LOD})\% \times \textbf{100}$$

Where,

Wsp = Weight of sample Wstd = Weight of standard

LOD = Loss on drying

**3. Method validation**

***3.1 System suitability***

For the system suitability testing five replicate of standard solution was prepared and sample s were also analyzed and % RSD was calculated.

**Table 1:** System suitability Luliconazole WS Specificity

| Sr. No | Test solution | Peak Abs. |
|---|---|---|
| 1 | Std1 | 0.5289 |
| 2 | Std2 | 0.5283 |
| 3 | Std3 | 0.5292 |
| 4 | Std4 | 0.5288 |
| 5 | Std5 | 0.5282 |
| | Average: | 0.52868 |
| | %RSD: | 0.07957813 |

### *3.2 Placebo preparation*

The excipients used in the formulation of Luliconazole in similar unit (aroung 5 gm ) ws prepared where, about 1 g was weighted and dissolved in around 70 ml of methanol. The solution was sonicated for 5 min and volume was made up to 100 ml with the same solvent. The solution was filtered through whatmann no 4. Standard and sample solution was prepared as similar method and the absorbance was compared between placebo, standard and the sample solution to see the interference of the placebo in the sample.

Result: there was slightly interference of the placebo observed in the sample

### *3.3 Accuracy*

Placebo solution was prepared. 100 % standard solution was prepared in triplicates.

Three different concentrations (50%, 100% and 150% ) of sample solution were prepared by adding known amount of drug substance to placebo. All the sample solutions were prepared in triplicates. All the samples were analyzed and the % recovery was calculated along with the % RSD.

**Table 2:** Percentage recovery data of Luliconazole

| Percentage | Amount taken (std) in g | Amount taken in gm | % Recovered | Mean recovery (%) | % RSD |
|---|---|---|---|---|---|
| 50 | 0.0256 | 0.5233 | 99.86 | 99.29 | 0.695565 |
| | | 0.5250 | 98.52 | | |
| | | 0.5114 | 99.48 | | |
| 100 | | 1.025 | 98.32 | 98.97 | 0.893785 |
| | | 1.0115 | 98.62 | | |
| | | 1.0473 | 99.98 | | |
| 150 | | 1.5091 | 100.29 | 100.07 | 1.071868 |

### *3.4 Precision*

**a) System Precision (Repeatability)**

a. Six replicate of standard solutions was prepared
b. According to the procedure samples were analyzed
c. % RSD was calculated for the peak of the standard solutions

**Table 3:** System Precision

| Sr. No. | Test solution | Peak Abs. |
|---|---|---|
| 1 | Standard 1 | 0.5265 |
| 2 | Standard 2 | 0.5252 |
| 3 | Standard 3 | 0.5249 |
| 4 | Standard 4 | 0.5251 |
| 5 | Standard 5 | 0.5251 |
| 6 | Standard 6 | 0.5255 |
| | Average: | 0.52538 |
| | % RSD: | 0.110603 |

b) Intermediate precision ( Ruggedness)

a. Six replicate of standard and six individual sample solutions by three analysts was prepared on the first day keeping the other condition same.

b. According to the procedure samples were analyzed.

c. Second day again six replicate of the samples by three analysts was prepared keeping the other conditions same.

d. According to the procedure samples were analyzed.

e. The individual day and combined day area between three analyst was compared and showed in the table below.

| Analyst: A | Analyst: B | Analyst: C |
|---|---|---|
| Day: 1 | Day: 1 | Day: 1 |
| Day: 2 | Day: 2 | Day: 2 |

**Table 9**

**Analyst A:** Day 1

| **Sr. No.** | **Test Solution** | **Weight (g)** | **Peak Abs.** | **Assay (%)** |
|---|---|---|---|---|
| 1 | Standard | 0.0256 | 0.5288 | |
| 2 | Sample 1 | 0.5233 | 0.2721 | 99.86 |
| 3 | Sample 2 | 0.5250 | 0.2693 | 98.52 |
| 4 | Sample 3 | 0.5114 | 0.2649 | 99.48 |
| 5 | Sample 4 | 1.025 | 0.5247 | 98.32 |
| 6 | Sample 5 | 1.0115 | 0.5194 | 98.62 |
| 7 | Sample 6 | 1.0473 | 0.5452 | 99.98 |
| **Average %** | **99.13** | | | |
| **RSD %:** | **0.736642** | | | |

**Analyst B:** Day 1

| **Sr. No.** | **Test Solution** | **Weight (g)** | **Peak Abs.** | **Assay (%)** |
|---|---|---|---|---|
| 1 | Standard | 0.0256 | 0.52538 | |
| 2 | Sample 1 | 0.5278 | 0.2736 | 100.21 |
| 3 | Sample 2 | 0.5201 | 0.2714 | 100.87 |
| 4 | Sample 3 | 0.5253 | 0.2769 | 101.90 |
| 5 | Sample 4 | 1.011 | 0.5251 | 100.40 |
| 6 | Sample 5 | 1.009 | 0.5155 | 98.76 |
| 7 | Sample 6 | 1.0063 | 0.5106 | 98.09 |
| **Average %** | **100.04** | | | |
| **RSD %:** | **1.395737** | | | |

**Analyst C:** Day 1

| **Sr. No.** | **Test Solution** | **Weight (g)** | **Peak Abs.** | **Assay (%)** |
|---|---|---|---|---|
| 1 | Standard | 0.0257 | 0.5278 | |
| 2 | Sample 1 | 1.024 | 0.5222 | 98.51 |
| 3 | Sample 2 | 1.005 | 0.5176 | 99.49 |
| 4 | Sample 3 | 1.039 | 0.5396 | 100.32 |
| 5 | Sample 4 | 1.522 | 0.776 | 98.49 |
| 6 | Sample 5 | 1.5006 | 0.7791 | 100.29 |
| 7 | Sample 6 | 1.5012 | 0.783 | 100.76 |
| **Average %** | **99.64** | | | |
| **RSD %:** | **0.979314** | | | |

**Analyst A:** Day 2

| Sr. No. | Test Solution | Weight (g) | Peak Abs. | Assay (%) |
|---|---|---|---|---|
| 1 | Standard | 0.0256 | 0.5264 | |
| 2 | Sample 1 | 1.025 | 0.5256 | 98.93 |
| 3 | Sample 2 | 1.0115 | 0.525 | 100.14 |
| 4 | Sample 3 | 1.0058 | 0.5277 | 101.22 |
| 5 | Sample 4 | 1.0099 | 0.5284 | 100.95 |
| 6 | Sample 5 | 1.0130 | 0.5229 | 99.29 |
| 7 | Sample 6 | 1.0473 | 0.5322 | 98.04 |
| **Average %** | **99.76** | | | |
| **RSD %:** | **1.232595** | | | |

**Analyst B:** Day 2

| Sr. No. | Test Solution | Weight (g) | Peak Abs. | Assay (%) |
|---|---|---|---|---|
| 1 | Standard | 0.0256 | 0.529 | |
| 2 | Sample 1 | 0.5278 | 0.2766 | 100.61 |
| 3 | Sample 2 | 0. 5201 | 0.2678 | 98.85 |
| 4 | Sample 3 | 0.5253 | 0.2683 | 98.06 |
| 5 | Sample 4 | 1.011 | 0.5189 | 98.54 |
| 6 | Sample 5 | 1.009 | 0.5179 | 98.54 |
| 7 | Sample 6 | 1.0063 | 0.5201 | 99.23 |
| **Average %** | **99.76** | | | |
| **RSD %:** | **1.232595** | | | |

**Analyst C:** Day 2

| Sr. No. | Test Solution | Weight (g) | Peak Abs. | Assay (%) |
|---|---|---|---|---|
| 1 | Standard | 0.0257 | 0.528 | |
| 2 | Sample 1 | 1.024 | 0.5248 | 98.96 |
| 3 | Sample 2 | 1.005 | 0.5296 | 101.76 |
| 4 | Sample 3 | 1.039 | 0.5296 | 98.56 |
| 5 | Sample 4 | 1.522 | 0.7804 | 99.01 |
| 6 | Sample 5 | 1.5006 | 0.7855 | 101.08 |
| 7 | Sample 6 | 1.5012 | 0.7849 | 100.96 |
| **Average %** | **100.06** | | | |
| **RSD %:** | **1.36327** | | | |

**Table 4:** RSD between three analyst on two different days

| **Analysts** | **Day 1** | **Day 2** |
|---|---|---|
| A (%RSD) | 0.736642 | 1.232595 |
| B (%RSD) | 1.39737 | 0.900267 |
| C (%RSD) | 0.979314 | 1.36327 |
| **Mean % RSD** | **1.03723** | **1.16537** |
| **Total Mean % RSD** | **1.1013** | |

### *3.5 Linearity*

a) The sample solution of 80%, 90%, 100 % and 120 % concentration of sample weight were prepared.

b) According to the procedure the samples were analyzed.

c) The calibration curve where, concentration versus peak area of solution was plotted.

d) The correlation coefficient, slope, y-intercept from the calibration curve was determined.

**Table 5:** obtained Peak abs. at 296 nm

| Sr. No. | Concentration of Analyte | Peak Abs. at 296 nm |
|---|---|---|
| 1 | 80% | 0.4303 |
| 2 | 90% | 0.4957 |
| 3 | 100% | 0.5548 |
| 4 | 110% | 0.612 |
| 5 | 120% | 0.6831 |
| | Correlation Coefficient ($R^2$) | 0.9987 |

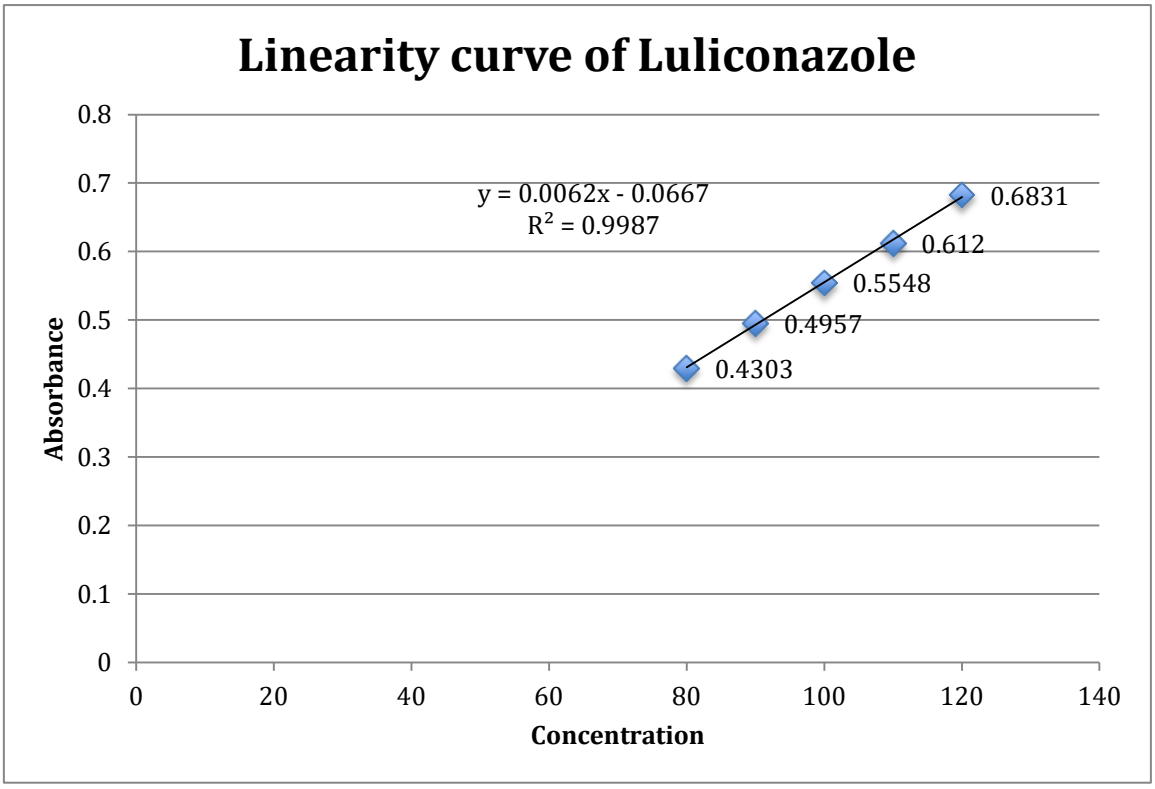


**Figure 1**

### *3.6 Robustness*

**a)** Three sample solutions were prepared according to the analytical method with minor deviations. The minor deviations was changed in wavelength ±1 nm.

**b)** The samples were analyzed according to the procedure and assay % was calculated.

**Table 6:** Change in wavelength: Abs, at 297 nm

| Sr. No. | Test solution | Weight (g) | Abs. | Assay ( %) |
|---|---|---|---|---|
| 1 | Standard | 0.0256 | 0.5296 | |
| 2 | Sample 1 | 1.0058 | 0.5233 | 99.77 |
| 3 | Sample 2 | 1.0099 | 0.5202 | 98.78 |
| 4 | Sample 3 | 1.0130 | 0.5264 | 99.65 |
| Average % | 99.40 | | | |
| % RSD | 0.543539 | | | |

**Table 7:** Change in wavelength: Abs at 295 nm

| Sr. No. | Test solution | Weight (g) | Abs. | Assay ( %) |
|---|---|---|---|---|
| 1 | Standard | 0.0256 | 0.5345 | |
| 2 | Sample 1 | 1.025 | 0.5271 | 99.06 |
| 3 | Sample 2 | 1.0115 | 0.524 | 98.68 |
| 4 | Sample 3 | 1.0473 | 0.5295 | 99.98 |
| Average % | 99.24 | | | |
| % RSD | 0.67355 | | | |

### *3.7 Range*

**Table 8**

| **Sr. No.** | **Concentration of Analyte** | **Peak Abs. at 296 nm** | **% Recovery** |
|---|---|---|---|
| 1 | 80% | 0.4303 | **101.78** |
| 2 | 90% | 0.4957 | 101.87 |
| 3 | 100% | 0.5548 | 100.18 |
| 4 | 110% | 0.612 | 99.05 |
| 5 | 120% | 0.6831 | 100.78 |
| | Mean % Recovery | 100.73 | |
| | Correlation Coefficent ($R^2$) | 0.9987 | |

## 4. Result and Discussion

A simple and reliable method has been developed for the determination of assay of Luliconazole in semisolid dosage formulation. Beers law was followed in concentration range of 80 %-150 % for Luliconazole at 296 nm in methanol and water. Correlation coefficient ($R^2$) was found to be 0.9987. The precision was studied for

System Precision, i.e., Repeatability (% RSD less than 2 %, i.e., 0.9987) and Intermediate precision, i.e., Ruggedness with three analyst for two different days keeping other conditions same and it is concluded that % RSD, Mean % RSD, Total Mean % RSD was found in the range of 0.7366-1.3957, 1.03723-1.16537, 1.1013 respectively which indicates that method is reliable and very likely to produce the same and predictive results. Accuracy of the method was determined by calculating the mean % recovery at 50 %, 100 % and 150 % level and found in the range of 98.97 to 100.07 %. Range was determined by calculating Mean % Recovery (i.e., 100.73) and Correlation Coefficent (i.e., $R^2$= 0.9987) at 80-120 % concentration of Luliconazole which indicates that set method is precise, accurate and linear at 80-120 % concentration level. Robustness was studied by slightly changing the wavelength i.e. ± 1nm and results indicate that the method remains unaffected by small variation in wavelength.

## 5. Conclusion

The observation and results obtained from the validation study clearly indicated that the developed analytical method is accurate, precise, specific and linear. Since all the result are within the limit, the above stated analytical method is validated as per the ICH guidelines and can be employed for routine analysis of Luliconazole in semisolid dosage form. In regard to constraint/limitations of the study, slight interference of the placebo in the sample was observed which affects the specificity of the method. Study was conducted using methanol only as solvent, however, Luliconazole is soluble in other organic solvents as well such as ethanol, dimethyl formide.

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